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The diversity and radiation of the largest monophyletic animal group on New Caledonia (Trichoptera: Ecnomidae: *Agmina*)

M. ESPELAND*† & K. A. JOHANSON*

**Department of Entomology, Swedish Museum of Natural History, Stockholm, Sweden*
†Department of Zoology, Stockholm University, Stockholm, Sweden

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Abstract

In area, New Caledonia is the smallest of the world's 25 official biodiversity hotspots, but in many taxonomic groups, the island has the highest concentration of species on earth, particularly so in the freshwater insect order Trichoptera. This study aims at applying molecular data and morphology for estimating the real species diversity of the genus *Agmina* on New Caledonia and investigating potential effects of ultramafic rock substrate on diversification. A dated molecular phylogeny was applied to study diversity and diversification related to geological substrate using the dispersal–extinction–cladogenesis model, DIVA and Bayesian ancestral character reconstruction. More than 47 species (> 63%) were unknown to science. Initial radiation occurred on ultramafic substrate followed by several independent dispersal events to nonultramafic substrate. The rate of shift from ultramafic to nonultramafic substrate was significantly higher than the rate of shift in the opposite direction, indicating a possible cost associated with living on ultramafic substrate.

Introduction

Despite several hundred years of study, most of the world's biodiversity remains unknown and the estimates of the world's total species diversity vary by more than a magnitude (Wilson, 1992). A large proportion of organisms are still undescribed and thus unavailable for biological studies. This lack of knowledge [the Linnean shortfall (Brown & Lomolino, 1998)] prevents important progress in biological sciences and in the protection of the species (Monaghan *et al.*, 2009). Furthermore, for most taxa, the distribution is not adequately known whether on global, regional or local scale [the Wallacean shortfall, (Lomolino, 2004)], which is of serious hindrance when, e.g. planning which areas to protect (Polasky *et al.*, 2000; Gaston & Rodrigues, 2003).

New Caledonia, situated in the south-western part of the Pacific region, is the smallest of the world's biodiversity hotspots (Myers *et al.*, 2000). It has been shown that the island has an exceptionally high species diversity of various organisms, and both the macro and micro-endemism is high (e.g. Chazeau, 1993; Morat, 1993; Lowry, 1998; Murienne *et al.*, 2005, 2008; Oláh *et al.*, 2006; Espeland *et al.*, 2008; Grandcolas *et al.*, 2008; Johanson & Keijsner, 2008; Malm & Johanson, 2008; Swenson *et al.*, 2008; Espeland & Johanson, 2010). Around one-third of the surface of the main island Grande Terre is covered by ultramafic rock substrate very rich in heavy metals and low in nutrients (Guillon, 1969; Trescases, 1969). These areas have particularly high diversity and endemicity of organisms, especially plants (Brousemiche, 1884; Jaffré & Latham, 1974; Lowry, 1991; Jaffré, 1992; Morat, 1993), but their intactness are also endangered by, e.g. mining and frequent fires (e.g. Lowry, 1998; Pascal *et al.*, 2008).

As the Trichoptera (caddisflies) have aquatic larvae, which are generally sensitive to pollution (Resh & Rosenberg, 1984; Rosenberg & Resh, 1993), and because of the high concentration of heavy metals on large parts of the island, they are unexpected to be diverse on New Caledonia. Despite this, caddisflies are exceptionally diverse on New Caledonia (Espeland & Johanson, 2010). One of us (KAJ) estimated the total caddisfly richness of New Caledonia to around 600 species, which with nearly

Correspondence: Marianne Espeland, Department of Entomology, Swedish Museum of Natural History, PO Box 50007, SE-104 05 Stockholm, Sweden. Tel.: +46 8 51954096; fax: +46 8 51954099; e-mail: Marianne.Espeland@nrm.se

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14 000 described species globally, is 4% of the world Trichoptera fauna. Only 178 New Caledonian species in the order are formally described. Twenty-seven species are described in the genus *Agmina* Ward & Schefter, 2000 (Ecnomidae), which is currently the third largest genus of New Caledonian caddisflies, only surpassed by the *Helicopsyche* (30 species, Johanson, 1999) and the *Orthopsyche/Caledopsyche/Abacaria* complex (around 28 species, Espeland & Johanson, 2010). Cockerell (1929) stated that the freshwater fauna on New Caledonia is limited. In contradiction, Ward (2003) demonstrated that almost 40% of the currently described *Agmina* species are known from single specimens and hypothesized that the number of species is probably substantially higher than what is currently known.

Agmina is an excellent model group for investigating how much of the insect fauna remains to be discovered on New Caledonia; and how the diversification of species was related to the presence of ultramafic rock substrate across time. The test of Ward's (2003) hypothesis of species diversity is performed by species limitations based on morphology *sensu* Ward & Schefter (2000) and Ward (2003), and a phylogeny model derived from molecular data. To study the substrate association, we apply historical biogeographical methods and Bayesian ancestral character reconstruction on a dated phylogeny.

Materials and methods

Sampling and data

Material from 156 localities on New Caledonia was sampled during the years 2001–2006 and fully available for this study. The ecnomids were sorted to species based on male genitalia and wing morphology following Ward & Schefter (2000) and Ward (2003). If available, at least three specimens of each species or morphospecies from widespread locations were included in the data set. Members of all other Ecnomidae genera were included as near outgroups. Representatives of the two Polycentropodidae species *Neureclipsis bimaculata* (Linnaeus, 1758) and *Polycentropus irroratus* (Curtis, 1835) were included as distant outgroups. The phylogeny was rooted with the hydropsychid species *Orthopsyche pakaha* Oláh & Johanson, 2006. The outgroups were selected based on Johanson & Espeland (2009). The complete data set contained 200 taxa, of which 193 had at least partial DNA sequences fore each gene. Locality information and voucher codes for all specimens can be found in Table S1 of the supporting information.

Molecular methods

The molecular data set for the phylogenetic analyses included one mitochondrial gene sequence fragment [cytochrome oxidase I (COI)] and three nuclear gene sequence fragments [elongation factor 1-alpha (EF-1a), cadherin-like gene (CAD) and RNA polymerase II (RP2)] generated following the extraction, PCR and sequencing protocols in Espeland & Johanson (2010) and Johanson & Malm (2010), with only few exceptions: For the second half of the EF-1a fragment, the forward primer EcnoIF (5′ TACATCAAGAAGATCGGNTACAACC 3′, this work) was used instead of the primer M46.1 by Whiting (2002), with an annealing temperature of 53 °C. This primer worked especially well for the Ecnomidae, Polycentropodidae, Hydropsychidae and Xiphocentronidae. The COI gene fragment was sequenced in two halves fore some specimens, with the primers LCO (forward, Folmer *et al.*, 1994) and MIDR (5′ GAACHGGATGAACWGTNT AHCCNCC 3′, reverse, annealing temp. 51 °C, this work) for the first half, and MIDF (5′ GAGCACWGATA TAGCHTTYCCYCG 3′, forward, annealing temp. 51 °C, this work, and HCO (reverse, Folmer *et al.*, 1994) for the second half. These two internal COI primers work well for a wide range of caddisflies (Strandberg & Johanson, in press). Sequences new to this study have GenBank accession numbers GU966688–GU967373. All other sequences are taken from Johanson & Espeland (2009) and Espeland & Johanson (2010). None of the sequences in this study has any shifts in reading frame or contain stop codons, which would indicate amplification of pseudogenes.

Phylogenetic analysis

The phylogenetic analyses were run on six different data sets: individual COI, EF-1a, CAD, RP2, nuclear gene fragments combined and nuclear + mitochondrial gene fragments combined. At least partial sequences were present for all specimens for EF-1a, whereas three sequences were missing entirely for COI and CAD, and six sequences were missing entirely for RP2.

Bayesian phylogenetic inference analyses were executed using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) as implemented on the Bioportal cluster, University of Oslo (http://www.bioportal.uio.no). Analyses were run for 20 000 000 generations for each single-gene data sets, and for 40 000 000 generations for all nuclear genes combined and all nuclear + mitochondrial genes combined. In all analyses, trees were sampled every 2000th generation. The combined gene analyses were partitioned by gene and/or codon position. The single-gene analyses were unpartitioned or partitioned by codon position. Appropriate substitution models were selected in MrModeltest v. 2.3 (Nylander, 2004) under the Akaike criterion. Model parameters were unlinked across data partitions, and the prior for rate variation among partitions was set to variable. For all analyses, several runs with different parameter combinations were performed to assess convergence. The temperature parameter lambda varied from 0.05 to 0.30 and the number of cold chains from three to six. Convergence for all data sets was assured, and results from several runs

were consistent when using a lambda of 0.12 and one hot and three cold chains. This combination was used in all subsequent analyses. After careful assessment of convergence (high ESS values and smooth curves on marginal density plots) in Tracer v. 1.5 (Rambaut & Drummond, 2007), the first 25% of the samples were discarded as 'burn-in'.

Parsimony analyses on separate gene data sets and combined data sets were executed in TNT (Goloboff *et al.*, 2004) using sectorial search (2000 iterations), with one round of parsimony ratchet and branch swapping per sectorial iteration, and one additional round of ratchet and branch swapping of all trees at the end. Clade support was given as Jackknife values (Farris *et al.*, 1996) with 2000 replications and a deletion probability of 36% for each character. The support was shown as GC values, which takes into account the percentage of groups contradicting the most frequent group (Goloboff *et al.*, 2004).

Delimitation of species based on molecular data was performed using the general-mixed Yule coalescent model (GMYC, Pons *et al.*, 2006) on the COI fragment.

Estimating divergence times

There are no known fossils in the family Ecnomidae, and for calibrating the phylogeny, we added several non-Ecnomidae taxa to the original data set having fossil representatives, i.e. the closely related families Polycentropodidae (*Neureclipsis* spp.) and Hydropsychidae (*Hydropsyche viduata* Ulmer, 1912 and *Potamyia nitida* Ulmer, 1912) (Table S1 in supporting information). These are Baltic amber fossils dated to 43.6–52.0 my (Ritzkowski, 1997; Weitschat & Wichard, 2002) and were implemented as a lognormal prior on the respective node heights (stem groups) with a mean of 0, standard deviation of 1 and an offset of 43.6 (shown as asterisks on nodes in Fig. 2). The tree prior was set to a Yule process and the rest of the priors were kept as default. Divergence times were estimated using an uncorrelated lognormal relaxed clock model (Drummond *et al.*, 2006) under a General time reversible model with gamma + invariants sites in beast 1.5.2 (Drummond & Rambaut, 2007) on the BioHPC cluster at Cornell University. Substitution and clock model parameters were unlinked between genes, and the topology was constrained to match the topology retrieved from the prior analyses in MrBayes. Two runs of 40 million generations, with sampling every 2000 generations, were run for each analysis, and the results were examined in Tracer 1.5 (Rambaut & Drummond, 2007). The two runs were combined using LogCombiner (distributed as part of the beast software package).

Geological substrate

To infer whether the *Agmina* radiation was affected by ultramafic or nonultramafic substrate, we used the dispersal–extinction–cladogenesis (DEC) model as implemented in Lagrange (Ree & Smith, 2008). This model estimates the inheritance of ancestral areas by daughter lineages, and the evolution of geographic ranges both at nodes and along branches is modelled. Analyses using dispersal–vicariance analysis in the software diva 1.1 (Ronquist, 1996) were performed for comparison of results with earlier studies. To account for uncertainty in topology, branch lengths and ancestral state reconstruction, 1000 random trees were drawn from the posterior distribution from beast after discarding burn-in. These trees formed the input data in the Multistate module of BayesTraits 1.0 (Pagel *et al.*, 2004) applied to seed a gamma distribution from a uniform distribution for the rate coefficients under a reversible jump Markov Chain Monte Carlo (MCMC) procedure and a hyperprior (0, 10) approach. BayesTraits was run for 40 million generations, and ancestral states were estimated for 10 nodes (Fig. 3) that received high support [e.g. posterior probability (PP) = 1] in the initial phylogenetic analyses. The first 10 million generations (25%) were discarded as burn-in. The results from BayesTraits were then compared with the results generated by the approaches in Lagrange and diva. To test whether a higher cost is associated with living on ultramafic substrate than on nonultramafic substrate, we inferred whether the rate of shift from ultramafic to nonultramafic substrate was higher than the rate of shift in the opposite direction using a likelihood approach in the Multistate module of BayesTraits on the 1000 random trees from the posterior distribution from beast. We performed two runs: (1) rates of substrate shift constrained to be the same; (2) rates were allowed to vary. The mean likelihood of substrate shift rates was calculated for each run, and significance was evaluated using a likelihood ratio test. A separate measure of the successfulness of diversification on ultramafic and nonultramafic substrate was calculated as the number of phylogenetic lineages on a particular substrate type over time in 1.0 million intervals, using age estimates and distribution patterns from the prior beast and DEC analyses above. In cases with individual taxa being present on both substrate types, we included lineage counts in both substrate alternatives.

Results

Phylogenetic analyses

Data characteristics and statistics from the parsimony analyses are given in Table 1. All clades with high Jackknife values (Jac) also received high PP. Analyses of separate genes did not reveal any strongly supported incongruence and all analyses seemed to be little affected by partition schemes so we discuss the combined analysis partitioned into genes only (Fig. 1). Two described genera of ecnomids are present on New Caledonia (Fig. 1); *Agmina*, a large, well-supported monophyletic

Table 1 Tree statistics for the different data sets. Missing indicates missing characters, Nchars is the total number of characters, constant is the number of constant characters, informative indicates the number of informative characters, No. trees is the number of trees recovered in parsimony analyses, and length is the length of the parsimony trees. CI is the consistency index and RI is the retention index.

Partition	Missing	NChars	Constant	Informative	No. trees	Length	CI	RI
COI	3	658	306	319	648	4000	0.155	0.731
EF-1a	0	1099	723	317	50 000+	1895	0.317	0.787
CAD	3	850	446	361	50 000+	2571	0.273	0.771
RP2	5	772	496	263	50 000+	1853	0.253	0.783
nDNA	0	2721	1665	941	50 000+	6486	0.273	0.771
Combined	0	3379	1971	1260	3360	10 607	0.23	0.752

COI, cytochrome oxidase I; EF-1a, elongation factor 1-alpha; CAD, cadherin-like gene; RP2, RNA polymerase II.

group (PP = 1, Jac = 100) and a smaller monophyletic group forming an undescribed genus *Caledomina* (K.A. Johanson, submitted) (PP = 1, Jac = 100). The genus *Ecnomina* is polyphyletic, with the Tasmanian *Ecnomina legula* Neboiss, 1977 being sister to *Caledomina* (PP = 1, Jac = 94), whereas another *Ecnomina* clade forms the sister group to *Agmina* + *Caledomina* + *E. legula*. This topology is retrieved in the Bayesian and the parsimony analysis but has high support only in the former (PP = 0.96); the Jackknife tree is incompletely resolved at sites where the branches of the Bayesian tree are short. *Ecnomina krokale* is sister species to the New Caledonian clades and the rest of the *Ecnomina* species (PP = 1, Jac = 100). The topology of the other Ecnomidae genera is largely consistent with the phylogeny of Johanson & Espeland (2009). In the Bayesian analysis, *Agmina* divides into the two major clades a and b (Fig. 1). Clade a is recovered in the parsimony analysis, whereas clade b that has short branches at deep levels in the Bayesian phylogeny is not supported in the parsimony analysis.

Divergence times

A time-calibrated chronogram is shown in Fig. 2. *Caledomina* split from the ancestor of *Caledomina* and the Australian *E. legula* around 25 Ma [CI (95% highest posterior density region): 21.4–38.2 Ma]. *Agmina* split from the ancestor to *Caledomina* and *E. legula* around 36 Ma (CI: 29.7–48.3 Ma). According to the molecular phylogeny, the diversity of *Agmina* species we observe today is a result of lineage splitting that occurred mostly in the Miocene and Pliocene, whereas *Caledomina* radiated in the late Miocene and in the Pliocene. As with all fossil-calibrated age estimates, the divergence times presented here are minimum ages.

Substrate and biogeography

According to our results (Fig. 3), *Agmina* caddisflies originated on ultramafic substrate, and subsequently dispersed into nonultramafic substrate (around 16–17 Ma according to DIVA and BayesTraits optimizations). All three methods applied give the same results for the *Agmina padi*–*Agmina kapiwa* clade (clade a) with early diversification on ultramafic substrate. In the *Agmina* sp. 53–*Agmina jepiva* clade (clade b), however, the DIVA and BayesTraits optimizations give the same results as for clade a, whereas in the DEC model, there is a dispersal from ultramafic to nonultramafic substrate already in the ancestor of clade b, and the early radiation of the *Agmina /i> sp. 53–Agmina* sp. 26 clade (clade c) occurred on nonultramafic substrate.

Diversity, substrate and endemism

In the New Caledonian material, 47 *Agmina* species were recognized as new to science based on morphology (features of male genitalia, sensu Ward & Schefter, 2000) and around 53 based on the molecular phylogeny (based on GMYC, not shown), i.e. more than 63% of the extant species are un-described. The new species will be described in subsequent papers. In addition, 22 of the 27 previously described species were re-collected from one or more of the 156 recently sampled localities. Four species of the undescribed genus *Caledomina* were found. Not all morphological species conform to the molecular species (Fig. 1). *Agmina kara* Ward and *Agmina vuegi* Ward are paraphyletic with respect to each other, and *A. kapiwa* is paraphyletic unless *Agmina* sp. 56 is included. *Agmina mariae* forms a complex of cryptic species that started diverging around 6 Ma. *Agmina nodosa* and *Agmina* sp. 4, as well as *Agmina diriwi* and *Agmina* sp. 25, are morphologically easily distinguishable based on male genitalia, but are genetically almost identical. Other apparent species complexes are *Agmina* sp. 24 and *Agmina* sp. 31 that appear to have diverged almost 5 and 2 Ma, respectively.

Many *Agmina* species have a restricted distribution, and 21 species are only known from a single specimen and/or locality (Table S1 of the supporting information).

The endemism is high both on ultramafic (84%) and nonultramafic (71%) substrate (Fig. 3). Only 8 of the about 80 recognized *Agmina* species are found on both substrate types. The DEC model suggests 12 dispersal events from ultramafic substrate to nonultramafic substrate, and 6 the other way around. The most parsimo-

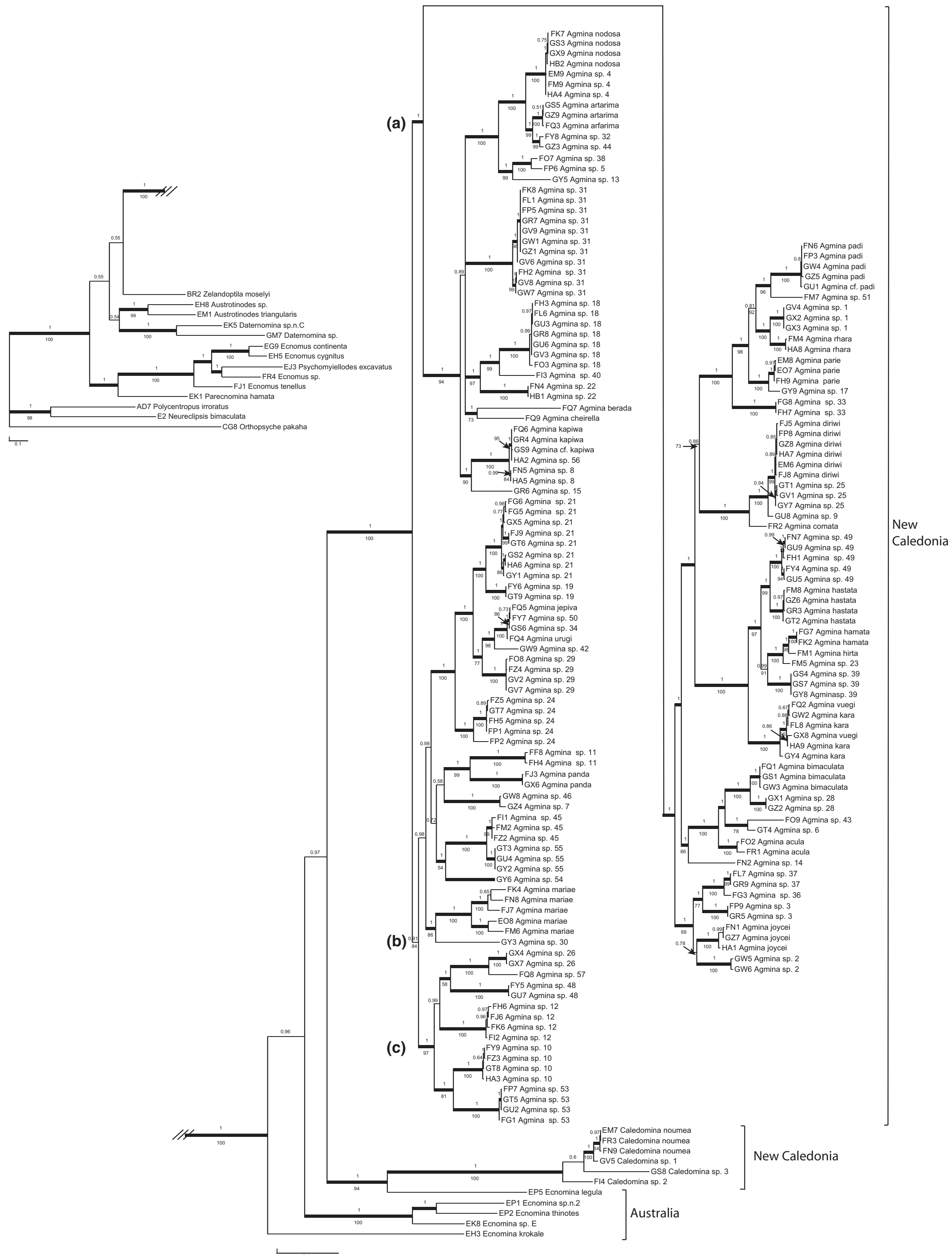

Fig. 1 Phylogenetic relationships of the New Caledonian Ecnomidae caddisflies inferred from Bayesian analysis of mitochondrial and nuclear markers. Numbers above branches are posterior probabilities (PP) and below branches are Jackknife GC values. Thick branches indicate clades with a PP of 1 and/or a Jackknife value of 100. a–c are clades discussed in the text.

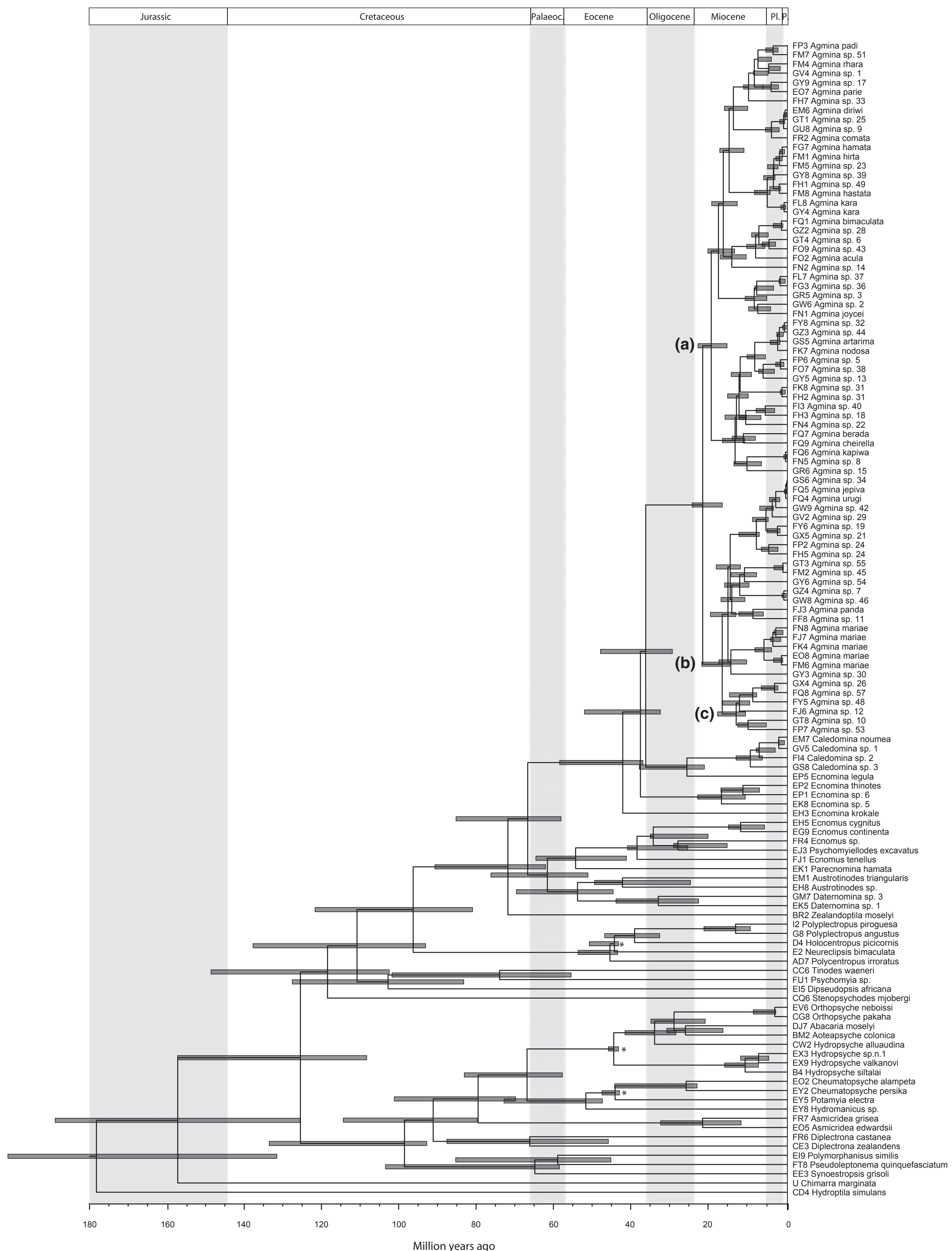

Fig. 2 Chronogram inferred from Bayesian analyses in BEAST. Asterisks indicate the nodes with fossil calibrations, and bars on nodes give the credibility intervals on ages. a–c are clades discussed in the text.

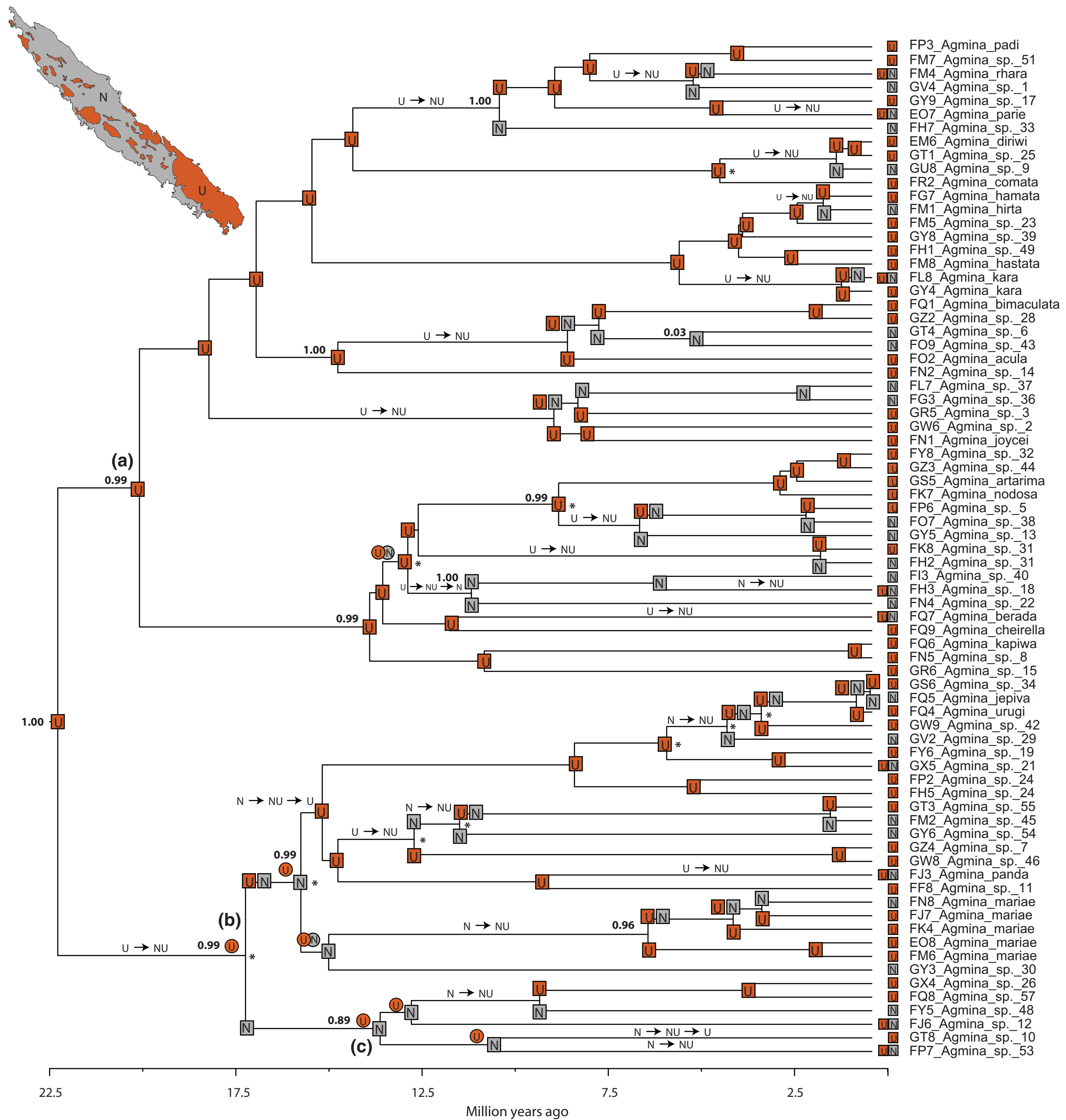

Fig. 3 Biogeographical analysis (DEC and DIVA) and ancestral state reconstruction of geological substrate. The map shows the distribution of ultramafic (orange, U) and nonultramafic (grey, N) substrate on New Caledonian Grande Terre. Boxes on internal branches indicate ancestral areas (with the highest likelihood) inherited by daughter lineages in the DEC model. If both the daughter lineages inherit the same ancestral area, the box is placed on the node, and if they inherit different areas, the appropriate boxes are placed on the branches to the left and right of the nodes. Asterisks denote nodes where alternative reconstructions of ancestral areas are within a probability of 0.1 of the reconstruction with the highest likelihood. Circles indicate the ancestral reconstructions from the DIVA analyses when incongruent with the results from the DEC model. The numbers in bold at certain nodes indicate the proportion of the likelihood associated with ultramafic substrate at that node as inferred by BayesTraits. a–c are clades discussed in the text. DEC, dispersal–extinction–cladogenesis.

nious result from the DIVA analysis, however, requires 23 dispersals from ultramafic substrate to nonultramafic substrate, and only two from nonultramafic substrate to ultramafic substrate. The BayesTraits analysis generally supports the results obtained from the DIVA analysis at the nodes where DEC and DIVA are incongruent.

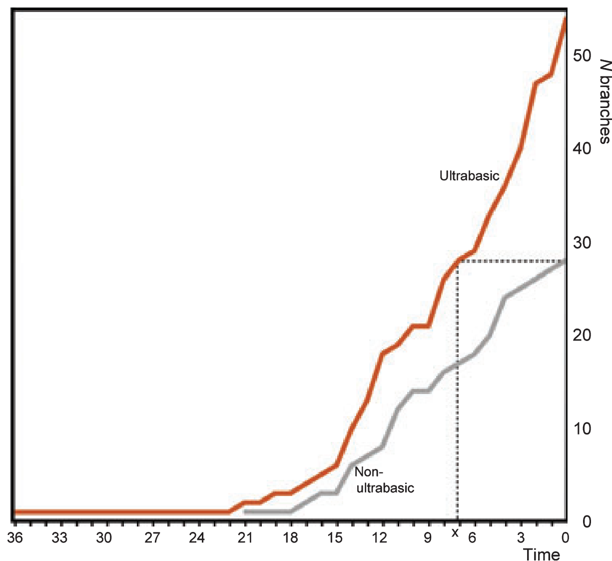

Fig. 4 Minimal number of phylogenetic lineages on ultramafic (orange) and nonultramafic (grey) substrate over time in 1.0 million intervals that is required to explain the observed modern distribution pattern. In cases with individual taxa being present on both substrate types, lineage counts in both substrate alternatives were employed. The 'X' on the time axis represents the time when the number of lineages on ultramafic substrate matched that of the actual number of lineages on nonultramafic substrate observed today (28 lineages), indicating that the number of lineages on ultramafic substrate predates those on nonultramafic substrate by approximately 7.2 million years.

According to the likelihood ratio test, the rate of shift from ultramafic substrate to nonultramafic substrate was significantly higher ($\chi_1^2 = 47.55$, $P \approx 0$) than the rate of shift from nonultramafic substrate to ultramafic substrate.

The number of phylogenetic lineages over time in 1.0 million intervals differed between ultramafic and nonultramafic substrates (Fig. 4). Subsequent to the initial detected radiation of the ancestral *Agmina* about 22 million years ago, consistently more lineages were present on ultramafic substrate compared to on nonultramafic substrate. The modern *Agmina* diversity is represented by 55 lineages (73%) on ultramafic substrate and 28 lineages (37%) on nonultramafic substrate, the latter representing a lineage number equal to that on ultramafic substrate more than 7 million years ago (X in Fig. 4). Over time, the increase of lineages on ultramafic substrate is found to be higher than on nonultramafic substrate.

Discussion

Phylogeny

Most clades are recovered in both the parsimony analyses and the Bayesian analyses. The few incongruent topologies are poorly supported in all analyses and are characterized in having short branches. For that reason, it is suggested that the incongruence at these levels of the phylogeny is a result of poor signal in the data set. The early radiation into two *Agmina* clades has probably been rapid, with little time for acquiring synapomorphies in the period between splits, as discussed by Whitfield & Kjer (2008). *Ecnomina* is recovered as paraphyletic in our phylogenetic hypothesis, which is supported by both morphological data (Cartwright, 2008) and molecular data (Johanson & Espeland, 2010).

Discrepancies between morphological and molecular species are common (Funk & Omland, 2003) and have also been observed in this study. Paraphyletic species might be expected in island radiations where speciations are the result of colonizations of new habitats (Balke *et al.*, 2007). Cryptic species are found also in other New Caledonian caddisfly groups (Espeland & Johanson, 2010), and further studies of species limits and speciations in New Caledonian caddisflies are desired to fully understand what processes are involved. Depending on method of species delimitation, around 80 species of *Agmina* are currently recognized from New Caledonia, more than 63% of them still undescribed. This number is likely to rise with increased collecting effort because several areas on New Caledonia still remains unsampled, and only 22 of 27 earlier described species were re-sampled so far.

Biogeographical history and substrate

Our results suggest either one dispersal event of an ancestral ecnomid species from Australia to New Caledonia around 37 Ma followed by a dispersal from New Caledonia back to Australia around 25 Ma resulting in the modern *E. legula*. Alternatively, two separate dispersal events from Australia to New Caledonia affected the ancestors of *Agmina* and *Caledomina*, where the ancestor of *Agmina* came to New Caledonia around 22–36 Ma and the ancestor to *Caledomina* came to New Caledonia around 25 million years ago. We find the second option with two separate dispersal events more likely, because many New Caledonian groups have Australian ancestors (e.g. Ladiges & Cantrill, 2007), but to our knowledge, only two Australian taxa might have New Caledonian ancestors (Hardy, 1977; Lowry & Plunkett, 2010). An Australia–New Caledonia connection long after the break-up of Gondwana has been observed in other New Caledonian freshwater insects (Balke *et al.*, 2007) and concur with the hypothesis that New Caledonia was entirely submerged until around 37 Ma (Cluzel *et al.*, 2001; Murienne *et al.*, 2005).

The results from the analyses of substrate association largely agree with our previous studies on the effects of the ultramafic substrate on New Caledonian caddisfly radiations (Espeland *et al.*, 2008; Espeland & Johanson, 2010). The results derived from the three methods DEC,

DIVA and BAYESTRAITS mainly correspond, but there are some discrepancies in reconstruction of ancestral areas between the DEC model and the two other methods. This is explained by differences in how the competing methods reconstruct the ancestral distributional states. Both DIVA and BAYESTRAITS search for ancestral distributions, whereas DEC estimates ancestral distributions inherited by daughter lineages (Kodandaramaiah, 2010).

The ultramafic substrate has been very important in early species radiations and still is very important, with more than half of the currently known species occurring only on this substrate, even though it only covers around one-third of the island. Shifts from ultramafic substrate to nonultramafic substrate occur at a significantly higher rate than from nonultramafic substrate to ultramafic substrate, indicating that adaptation to ultramafic substrate is associated with a cost, probably at the egg, larval or pupal developmental stage because by living in water, those stages are more directly tied to substrate quality. As early developmental stages of New Caledonian Trichoptera are informative for phylogeographic and phylogenetic analyses (Johanson, 2007), these should be included in future studies of the evolution of the caddisfly fauna of the island. The number of *Agmina* lineages on ultramafic substrate was found to be higher than on nonultramafic substrate over time since the initial radiation about 22 million years ago. However, the number of lineages over time plotted and available in this study (Fig. 4) only takes into consideration ancestral lineages needed to explain the distribution of the modern diversity. The curve shapes over time are therefore assumptions, and not based on realistic models incorporating, e.g. lineage extinctions.

The New Caledonian ecnomid caddisflies

A heterogeneous island environment together with restricted dispersal abilities, and relative stability of habitats, might explain some of the exceptional diversity of *Agmina* and other genera (Espeland & Johanson, 2010) on New Caledonia. Caddisflies are poor dispersers compared with other groups (e.g. Petersen *et al.*, 1999), and adults are usually only found very close to water (Collier & Smith, 1997). Large differences in body size were interpreted as a possible adaptation to different feeding strategies in the New Caledonian hydropsychid caddisflies (Espeland & Johanson, 2010). Size differences are, however, subtle in the ecnomids (Ward & Schefter, 2000; Ward, 2003), thus other factors must be involved in the diversification of *Agmina*. Because *Caledomina* (undescribed) has only four recognized species but are much younger than *Agmina* with 80+ species, the difference in diversity in the two New Caledonian Ecnomidae genera is apparently proportional with the age difference. A large number of *Agmina* lineages were already present when the ancestor of *Caledomina* arrived, which could have left less available niches for the *Caledomina* radiation.

Chazeau (1993) listed 19 Trichoptera species from New Caledonia. As is often the case in the tropics, the low number of species reported by Chazeau and the large number of singletons reported by Ward (2003) were a result of grave under-sampling (e.g. Coddington *et al.*, 2009) as several hundred species of caddisflies are now known from the area (Espeland & Johanson, 2010). The *Agmina* diversity is to our knowledge the result of the largest species radiation of any animal group known to date from New Caledonia and surpassed by plants only by *Phyllanthus* (Euphorbiaceae) with which about 110 species is the largest plant genus on the island (Ulf Swenson, personal communication).

According to Cockerell (1929), the freshwater fauna of New Caledonia is '... necessarily somewhat limited, as the narrowness of the island, with a central mountain chain, causes the rivers to be small and short, …'. Chazeau (1993) supported Cockerell's opinion concluding that no representatives of the freshwater orders Plecoptera and Megaloptera, and only 19 Trichoptera species existed on the island. Ward (2003) on the other hand indicated that the fauna might be rich. As we demonstrated above and in previous works (Espeland *et al.*, 2008; Espeland & Johanson, 2010; and references therein), the New Caledonian freshwater fauna of the order Trichoptera is extremely rich. The small and short rivers with numerous unique microhabitats, together with the ultramafic substrate, may be involved in driving this diversity (Espeland & Johanson, 2010). Ward was right and Cockerell could not have been more wrong.

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Supporting information

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Table S1 List of specimens used in the study with voucher code and locality information.

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